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Short communication

Double strand DNA-templated copper nanoparticle as a novel fluorescence indicator for label-free detection of polynucleotide kinase activity

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ABSTRACT

Phosphorylation of DNA by polynucleotide kinase (PNK) takes an important role in DNA damage repair, replication and recombination. The evaluation of PNK activity has received an increasing attention due to the significance of PNK. Here, we present a label-free fluorescent method for PNK activity assay using double strand DNA (dsDNA) -templated copper nanoparticles (CuNPs) as a fluorescent indicator. Upon the PNK reaction, the dsDNA template is phosphorylated and then digested by λ exonuclease immediately, prohibiting the formation of fluorescent CuNPs due to the lack of dsDNA template. This homogeneous PNK activity assay does not require any other additional modifications of DNA substrate or complex design, making the proposed strategy simple, cost-effective and high throughput. The proposed strategy is sensitive, selective and exhibits a good assay performance in complex biological samples. The strategy presented here opens a new avenue for PNK assay and nucleic acid phosphorylation related research.

1. Introduction

Repair of DNA breakages which are commonly generated by irradiation or genotoxic agents is crucial in maintaining the integrity and functionality of the genome (Hoeijmakers, 2001). Most DNA repair pathways require DNA ligase to complete the repair process and the presence of 5′-phosphate and 3′-hydroxyl termini at break points are needed for ligation (Lindahl et al., 1999). Unfortunately, many chemically distinct groups are found at most break points (Caldecott, 2008; Alllinson, 2010). Therefore, a range of end-processing enzymes are indispensable for the modification of the termini of strand breaks, facilitating the efficient repair process (Izumi et al., 2000; Nitiss et al., 2006; Takahashi et al., 2007).

Polynucleotide kinase (PNK) is one of the most important end-processing enzymes and has been extensively studied in recent years (Chappell et al., 2002; Scheiienberg et al., 2011). It was reported that PNK could transfer the γ-phosphate of ATP to 5′-hydroxyl group of DNA/RNA molecules. The kinase activity of PNK makes the 5′-end of DNA substrate to be phosphorylated which plays an important role in DNA breakage repair, replication and recombination (Sobol et al., 2000; Koch et al., 2004; Horton et al., 2008). Additionally, PNK has been also widely used as an efficient tool in the field of molecular biology research (Frauendorf et al., 2003; Phillips et al., 2007). On account of the significance of PNK, the development of assays for PNK activities is therefore of fundamental importance.

The traditional detection methods for PNK are mainly based on radioisotope ^{32}P-labeling and polyacrylamide gel electrophoresis (Karimi-Busheri et al., 1998; Wang et al., 2001; Bernstein et al., 2005). However, these methods are time-consuming, costly and harmful to human health. Recently, some interesting assay strategies toward convenient detection of PNK activity were developed. For example, some FRET-based fluorescence assays for PNK using fluorescence dye labeled hairpin probes have been reported (Tang et al., 2005; Song et al., 2009; Wu et al., 2011). Based on marking DNA phosphorylation/biotinylation events with the fluorescent nanoparticles, a fluorometric assay of PNK activity was also described (Ma et al., 2010). However, these methods required fluorescence dye labeled DNA probes or biotin modified ATP analogs, which are costly and difficult to synthesize. Therefore, it is still highly desirable to develop facile label-free methods to monitor the PNK activity.

At present, using ultrasmall fluorescent metal nanoparticles as fluorescence markers have attracted a great attention in the field of biochemical analysis due to their excellent optical properties, facile surface modification and good biocompatibility (Biju et al., 2008; Shang et al., 2011). Here, we designed a simple, low cost and label-free assay to detect PNK activity using fluorescent

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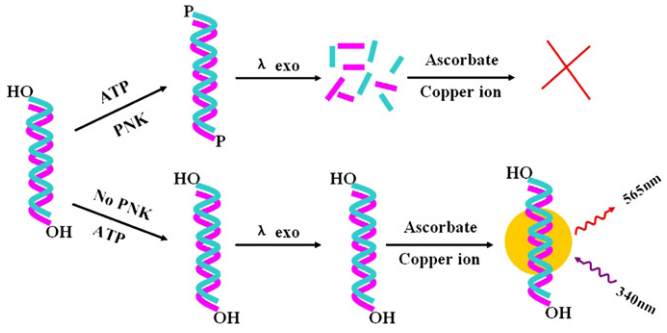
dsDNA-templated copper nanopaticles (CuNPs) as a fluorescence indicator. Fluorescent CuNPs using double-strand DNA (dsDNA) as templates were reported by Mokhir et al., and the CuNPs could not be formed using single strand DNA (ssDNA) templates (Rotaru et al., 2010). The obtained dsDNA-templated CuNPs exhibited excellent fluorescence properties and had been used as an ideal fluorescence probe (Zhou et al., 2011; Chen et al., 2012).

Using T4 Polynucleotide kinase (T4 PNK) as a model analyte, which was first discovered in 1965 as an RNA repair enzyme involved in the T4 bacteriophage infection process (Richardson, 1965), a CuNPs-based PNK detection method coupled with lambda exonuclease (λ exo) was developed. Lambda exonuclease is a highly processive 5′ to 3′ dsDNA exonuclease, which digests dsDNA with a phosphate moiety at 5′-ends and has very low activity on non-phosphorylated DNA (Mitsis et al., 1999). A dsDNA probe was introduced to act as both enzyme's substrate and template for CuNPs formation. After phosphorylation of dsDNA template by PNK in the presence of ATP, λ exo would digest dsDNA template immediately, prohibiting the formation of fluorescent CuNPs due to the lack of dsDNA template. Thus no obvious fluorescence could be detected. Compared to the previously reported FRET-based assays, this strategy does not require any fluorescence dye label or complex design. The present method is convenient with high sensitivity and selectivity. This new strategy may provide an alternative opportunity for PNK assay and biomedical research.



Scheme 1. Schematic illustration of the PNK assay based on the dsDNA-templated fluorescent CuNPs.

2. Experimental

2.1. Reagents and apparatus

The DNA templates used in this work were synthesized by Dalian Takara Bio Inc. (Dalian, China). The sequences of the DNA oligonucleotides were as follows:

P1: 5′-CATAGCGGCAGGATCAGTTACAGTG-3′
P2: 5′-CACTGTAACTGATCCTGCCGCTATG-3′

Lambda exonuclease (λ exo, 5 unit/μL), T4 polynucleotide kinase (T4 PNK, 10 unit/μL), uracil-DNA glycosylase (UDG, 5 unit/μL) and apurinic/apyrimidinic endonuclease 1 (APE 1, 10 unit/μL) were purchased from New England Biolabs (NEB, U.K.). Adenosine triphosphate (ATP), sodium ascorbate and lysozyme (41800 U/mg) were bought from Sigma-Aldrich (Shanghai, China). All other chemicals were of analytical grade. The enzyme reaction buffer contained 40 mM Tris-HCl (pH 7.4) and 20 mM $MgAc_2$. All solutions were prepared using ultrapure water, which was obtained through a Millipore Milli-Q water purification system (Billerica, MA) and had an electric resistance >18.2 MΩ.

The fluorescence measurements were carried out on an FL-7000 spectrometer (Hitachi, Japan). The fluorescence emission spectra of CuNPs were collected from 510 nm to 650 nm at room temperature with a 340 nm excitation wavelength.

2.2. Assay of PNK

In order to prepare dsDNA template, each ssDNA probe (P1, P2) was dissolved in enzyme reaction buffer and diluted to the desired concentration. Two ssDNA probes were mixed together and the mixture was then denatured at 95 °C for 10 min, and cooled slowly to room temperature.

The detection of PNK activity was carried out in 50 μL of enzyme reaction buffer containing 1 μM denatured dsDNA template, 1 mM ATP, 10 units λ exo and various concentrations of PNK. The mixture was incubated for 1 h at 37 °C. After incubation, 25 μL ascorbate (4 mM) was added and incubated for 30 min at 37 °C. Finally, 25 μL $CuSO_4$ (400 μM) was added into the solutions and the fluorescence spectra of formed CuNPs were recorded 3 min later. The solutions of PNK were diluted with a storage buffer consisting of 50% glycerol, 10 mM Tris-HCl (pH 7.4), 50 mM

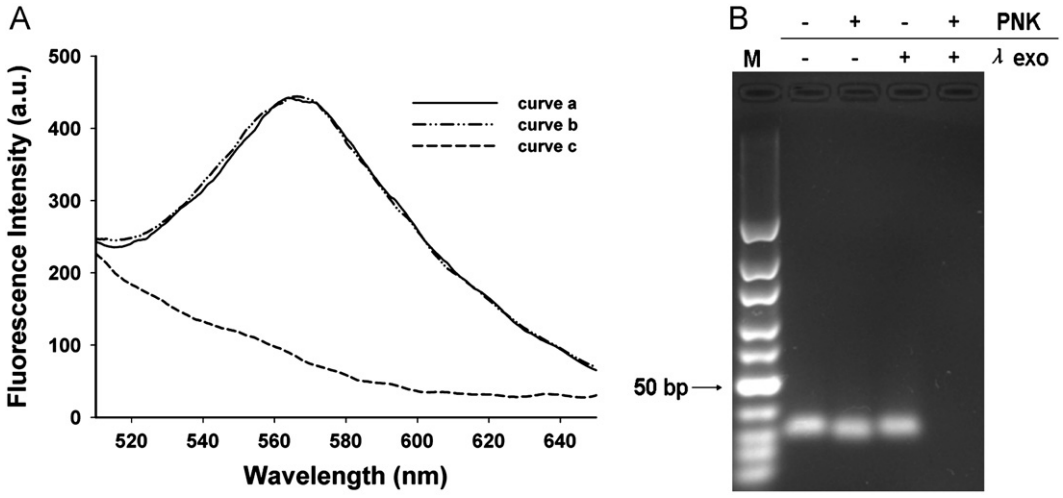


Fig. 1. (A) Fluorescence spectra of obtained CuNPs in the absence or presence of λ exo and PNK. (a) λ exo alone; (b) PNK alone; (c) PNK+λ exo. (B) Agarose gel (5%) electrophoresis images of dsDNA in the absence or presence of λ exo and PNK. The concentrations of λ exo, PNK and dsDNA were 10 U, 10 U/mL and 1 μM, respectively.